THE CONCENTRATION OF A NUTRILITE

FOR LEUCONOSTOC MESENTEROIDES

THE CONCENTRATION OF A NUTRILITE FOR LEUCONOSTOC MESENTEROIDES

DISSERTATION

Presented to the Faculty of the Graduate School of

The University of Texas in Partial Fulfillment

of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

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Acknowledgement

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CHAPTER I

INTRODUCTION

INTRODUCTION

Out of the study of the nutritional requirements of microorganisms has arisen the recognition of the unique role played by the "vitamin B complex". In contrast to the other vitamins, members of this group have been found essential not only to man and animals but to many species of bacteria, yeasts, and fungi as well. With the advent of this relationship between the important water-soluble group of vitamins and the nutritional requirement of microorganisms for them, a new approach to the problem of identifying members of the group became apparent. As a result, pantothenic acid, biotin, and folic acid were discovered, and in addition preliminary experiments with microorganisms led to the recognition of nicotinic acid, inositol, and p-aminobenzoic acid as members of the B family of vitamins.

The present investigation has to do with the use of the bacterium Leuconostoc mesenteroides. Snell[1] made observations indicating that yeast and liver extracts contained an active principle, not identical with any of the known nutrilites, which was necessary for the rapid growth of this organism. This study is an outgrowth of this initial observation.

Preliminary experiments showed that when seventeen compounds recognized as growth-stimulatory substances were added to a medium

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[1] E. E. Snell, private communication, January, 1941.

containing pantothenic acid[2], uracil[3], and guanine[3] in addition to amino acids, glucose, and inorganic salts, the rate of growth of Leuconostoc mesenteroides was found to be no greater than when they were omitted from the medium. On the other hand, growth was found to be definitely enhanced when small amounts of natural extracts were added. From this it was concluded that the substance (or substances) responsible for the rapid growth of the microorganism is either an unknown compound or one not yet known to cause growth stimulation of bacteria.

The procedures for concentrating this active principle are described in the main body of this report.

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[2] E. E. Snell, F. M. Strong, and W. H. Peterson, J. Bact., 38, 293 (1939).

[3] E. E. Snell and H. K. Mitchell, Proc. Nat. Acad. Sci., 27, 1 (1941).

CHAPTER II

EXPERIMENTAL

EXPERIMENTAL

Assay Method

The assay method used for determining the amount of the factor present in materials tested is similar to those developed in the laboratories of Dr. R. J. Williams for assays of the B vitamins.[4]

Organism.

Leuconostoc mesenteroides (Pd-60, American Type Culture Collection), the organism used, was carried in stab cultures in yeast extract glucose agar (1% glucose, 1% yeast extract, 1.5% agar) stored in the refrigerator. Inoculums for assays were prepared by transferring the organism from the stab culture to a tube of basal medium and allowing it to incubate from 14 to 18 hours at 37° C.

Basal Medium.

This is a modification of the basal medium used in the microbiological assay method for nicotinic acid developed by Snell and Wright.[5]

Acid hydrolyzed casein.— Fifty grams of vitamin-free (Labco) casein was mixed with 500 ml. of approximately constant boiling hydrochloric acid (1:1 concentrated hydrochloric acid and water) and allowed to reflux for 8-10 hours, and then concentrated in vacuo over the steam bath to a thick syrup. This was redissolved in water and concentrated again in the same manner. The syrup was dissolved in water, adjusted to a pH

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[4] University of Texas Publication, No. 4137, 1941.

[5] E. E. Snell and L. D. Wright, J. Biol. Chem. 139 675 (1941).

of 3.0 with sodium hydroxide solution, and shaken with 1 part of Darco G-60 per 10 parts of solids for 10 minutes and filtered. The filtrate was diluted with water to contain 100 mg. of dry matter per ml. and was preserved under toluene.

Adenine sulfate, guanine hydrochloride, and uracil solution.-- A solution of 1 mg. per ml. of each of these constituents was prepared by prolonged heating in the presence of a small amount of hydrochloric acid. The solution was stored in the refrigerator.

Vitamin supplement.-- A stock solution contained 0.6 γ per ml. of biotin and 200 γ per ml. of each of the following: thiamin, pyridoxin, nicotinic acid, and pantothenic acid. It was stored in the refrigerator under toluene.

Inorganic salts.-- Solution A contained 25 g. of potassium monohydrogen phosphate and 25 g. of potassium dihydrogen phosphate in 250 ml. of water.

Solution B contained 10 g. of magnesium sulfate heptahydrate, 0.5 g. of sodium chloride, and 0.5 g. of manganese sulfate tetrahydrate dissolved in 250 ml. of water. A few drops of concentrated hydrochloric acid were added and the solution was preserved under toluene. It was found that if the amounts necessary for assays were taken out by a syphon arrangement, thereby preventing the introduction of air into the solution, oxidation of the salts and their subsequent precipitation was avoided; the solution can be stored and used for an indefinite length of time.

Unsupplemented medium-- An unsupplemented medium of quadruple strength sufficient for 400 assay tubes was prepared by mixing together 200 ml. of hydrolyzed casein, 30 g. of sodium acetate trihydrate, 40 g. of glucose, 0.4 g. of tryptophane, and 0.4 g. cystine (the cystine was first dissolved in a small amount of dilute hydrochloric acid), and then diluting to about 800 ml. The solution was steamed in the autoclave for 10 minutes, filtered, and diluted to one liter. The medium was syphoned out as needed from under a layer of toluene to prevent its contact with air, and under these conditions could be kept indefinitely.

Basal medium.-- Sufficient medium, for example, for 10 assay tubes was prepared by mixing the following constituents:

Table I

Unsupplemented medium	25.0 ml.
Adenine, guanine, and uracil solution	1.0 "
Vitamin supplement	0.1 "
Salts A and B (each)	0.5 "

The resulting solution was adjusted to a pH of 6.8-7.0 and diluted to 80 ml.

Procedure.

Assays were performed in 6 inch bacteriological test tubes supported in a wire rack. Tubes which contained 0, 0.1, 0.2, 0.4, and 1.0 ml. of a solution of 1 mg. per ml. of "liver fraction B" were used to establish a standard curve (Fig. 1). Samples to be assayed were diluted

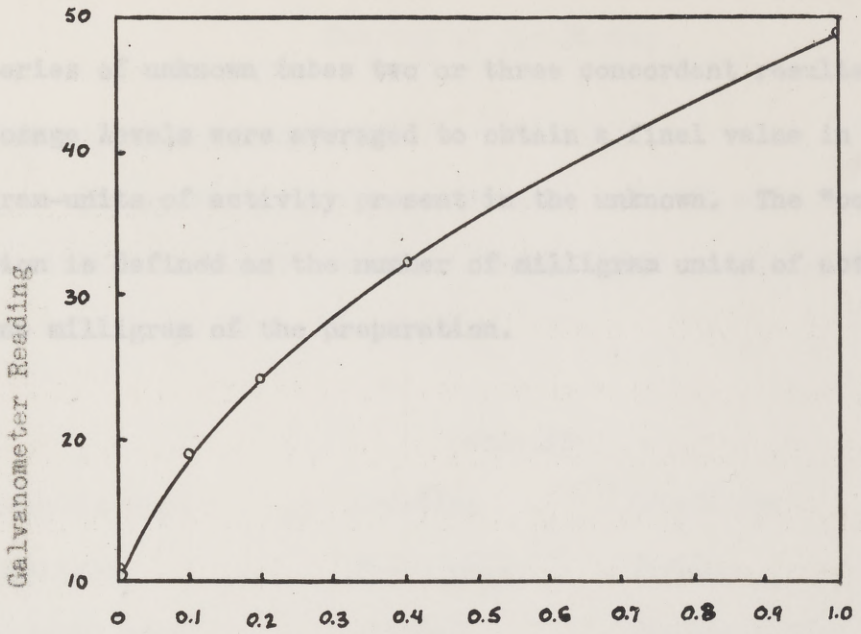

Fig. 1.

to contain approximately the equivalent of 1 mg. liver fraction B per ml. Usually four assay tubes were used per sample, containing 0.1, 0.2, 0.4, and 1.0 ml. of the above solution. All the tubes were then diluted to 2 ml., and 8 ml. of the basal medium was added. After autoclaving for 10 minutes and allowing the tubes to cool, each tube was seeded with one drop of a suspension of the test organism which had been washed by centrifuging with sterile water and resuspended. After a 12-14 hour period of incubation in a constant temperature water bath at 37° C. the turbidities produced by the growth of the organism were quantitatively measured in a thermoelectric turbidimeter.

The turbidities of the series of standard tubes were plotted versus the milligrams of liver fraction B present. The number of milligram-units (a milligram-unit = activity present in 1 mg. liver fraction B) in each of the unknown tubes was then read off the curve. For each

series of unknown tubes two or three concordant results at different dosage levels were averaged to obtain a final value in milligram- or gram-units of activity present in the unknown. The "potency" of a preparation is defined as the number of milligram units of activity present in one milligram of the preparation.

Sources of the Factor

Effect of Other Nutrilites.

The growth substances listed in Table II were tested in various quantities for their ability to cause rapid growth of the test organism and were found to be inactive.

Table II

Adenylic acid	Creatine	Riboflavin
Asparagin	Folic acid	Thymine
Ascorbic acid	Glycine	Traumatic acid
β-Alanine	Inositol	2-methyl-4-amino-α-naphthol (a synthetic vitamin K)
p-Aminobenzoic acid	Phytin	
Choline	Pimelic acid	Xanthopterin (anti-trout-anemia factor)

Relative Activity of Various Tissues.

Although no quantitative data were obtained, the amount of the factor in the different rat tissues listed below was found to decrease in the following order: liver, spleen, kidney, brain, muscle, and heart.

Amount of Factor Freed from Rat Liver by Autolysis and Enzymolysis.

Thirty minutes after its removal from the animal, 1 g. of rat liver was steamed to prevent autolysis. Its potency was found to be 0.3 (dry basis). When autolysis under toluene at 37° C. was allowed to take place for 48 hours the potency of another sample of the same liver was 0.8 (dry basis), a 2.7 fold increase in potency. Enzymolysis for 48 hours under toluene of fresh rat liver with a quantity of taka-diastase equal to one-half the weight of the liver increased the potency from 0.3 to

2.4 (dry basis). When taka-diastase was allowed to act on liver fraction B under toluene at 37° C. its potency increased from 1.0 to 1.1.

Potency of Natural Materials.

The following materials were assayed to determine relative amounts of the factor present: (The potency of liver fraction B was arbitrarily chosen as 1 mg. unit per mg.)

Table III

Substance	Potency (in mg.-units per mg. dry wt.)
Liver fraction B	1
Taka-diastase	0.7
Yeast extract (Difco)	0.4-0.9
Rice bran extract	0.3
Jack Bean meal (Arlco)	0.3
Alfalfa meal	0.2
Egg yolk	0.05
Whey	0.04
Egg white	0.03
Urine	0.03

Physical Properties of the Factor

Isoelectric Point.

In two determinations using four cups in a fractional electrical transport apparatus[6] the factor was found to have an isoelectric point of about 3.0.

Solubility.

Neither chloroform, ether, ethyl acetate, nor n-butyl alcohol was found to dissolve the factor from a solution of liver fraction B. Even a continuous extraction process yielded no activity in the organic solvent.

When to one part of a 10% aqueous solution of an active concentrate having a potency of about 50, 4 parts of the water miscible organic solvents listed below were added, the amount of activity precipitated was found to be as follows:

Table IV

Solvent added	Percent activity precipitated
Acetone	70
Ethyl alcohol (95%)	40
Dioxane	5
Dioxane + alcohol (1:1)	20

[6] R. J. Williams, J. Biol. Chem., 110, 589 (1935).

Soxhlet extraction of a dried and powdered eluate (pages 19 and 20 describe its preparation) potency 60 mg.-units per mg. using 1:1 dioxane and alcohol solution dissolved 90% of the activity. The potency of the filtrate was found to be 96.

Adsorption Characteristics.

Adsorption experiments were performed by adding the adsorbent to a clarified 4% aqueous solution of liver fraction B or of a concentrate of the factor. The conditions and results of the experiments are summarized in Tables V and VI.

Elution of Activity from Charcoal.

In each experiment 5 g. aliquots of Darco G-60 which had been mixed with solutions of liver fraction B, filtered, and dried, was suspended in 20 ml. of a 10% aqueous solution of the eluting agent. The resulting mixture was boiled under reflux for 10 minutes and filtered. The filtrates were assayed for activity after removing the aniline, pyridine, and phenol by shaking with ether. Barium ions were removed by adding sulfuric acid to the filtrates until no further precipitation took place. Table VII gives a summary of the results.

Table VII

Eluting agent	Aniline	Phenol	Pyridine	Barium hydroxide	Urea
Percent activity eluted	90	70	30	10	0

Table V

Darco G-60 as the Adsorbent

Potency of material treated, 1.0

Grams of adsorbent added per gram of dissolved solids	1.0	1.0	1.0	1.0	1.0	0.5	0.1	0.02
pH of solution	1.3	2.9	4.5	7.0	8.3	4.5	4.5	4.5
Percent adsorption	89	91	95	91	86	80	10	0

Potency of material treated, 5.0

Grams of adsorbent added per gram of dissolved solids	0.5	0.5	0.5	0.5	0.5
pH					
pH of solution	2.0	3.0	5.0	7.0	9.0
Percent adsorption	45	86	68	40	20

Table VI

Other Adsorbents

Adsorbent	"Super Filtrol"		Hydrous aluminum oxide gel
Potency of material treated	5.0	5.0	90
Grams of adsorbent added per gram of dissolved solids	4.0	4.0	1.0
pH of solution	1.0	3.0	5.0
Percent adsorption	82	72	80

Chemical Properties of the Factor

Destruction with Chemical and Physical Agents.

Alkaline hydrolysis.— No loss of activity took place upon autoclaving at 15 pounds pressure for 10 hours a solution of yeast extract in 1 N sodium hydroxide.

Acid hydrolysis.— Fifty percent of the activity was destroyed when yeast extract dissolved in 0.5 N sulfuric acid was autoclaved at 15 pounds pressure for 10 hours.

Nitrous acid.— When 400 mg. of yeast extract was dissolved in 15 ml. of 10% sodium nitrite and 5 ml. of 1 N sulfuric acid solution and allowed to stand for 24 hours at room temperature, a loss of activity amounting to 17% was found to have taken place.

Bromine.— Yeast extract in an aqueous bromine-saturated solution was observed to lose 53% of its activity after 2 hours.

Hydrogen peroxide.— Eighty-eight percent loss of activity occurred when yeast extract was allowed to stand for 24 hours in a 1% hydrogen peroxide solution.

Dry heat.— By keeping at 100° C. stoppered flask containing yeast extract for 24 hours, 40% of the activity was found to be destroyed.

Precipitation of the Factor with Metallic Ions.

By adding 10% aqueous solutions of silver nitrate, lead acetate, mercuric chloride, cadmium acetate, and ferric chloride to aliquots of

a 2% aqueous solution of a preparation (potency = 89) and assaying the supernatant liquids after centrifuging and precipitating the metallic ions with hydrogen sulfide, the silver, lead, and mercuric salts of the factor were found insoluble.

Esterification of the Factor.

Ethyl ester.— An absolute ethyl alcoholic suspension of a concentrate (potency = 80) of the factor was allowed to reflux in the presence of 0.3% sulfuric acid for 15 minutes. After neutralization 70% of the activity was extractable with ether. This activity was evidently in the form of an ester which was itself about 17% as active as the free acid obtained by subsequent hydrolysis. The remaining 30% of the activity was unextractable by ether and was presumably unesterified under the conditions used. Hydrolysis of the ester was accomplished by keeping it at 100° C. for 10 minutes in the presence of 1 N sodium hydroxide.

n-Butyl ester.— In order to find the time required for esterifying the factor with n-butyl alcohol, individual experiments were performed by adding 0.1 g. of a concentrate (potency = 80), 20 ml. of n-butyl alcohol and 0.06 ml. (0.3%) of concentrated sulfuric acid to each of five flasks. Their contents were then boiled under reflux for from 1 to 30 minutes, stopping the reactions by cooling quickly and adding 0.5 g. of dry sodium bicarbonate and 100 ml. of chloroform. The resulting chloroform solutions were washed 3 times with 50 ml. portions of water. Assays of both the aqueous and choloroform solutions were obtained, the latter by evaporating

off the organic solvents and hydrolyzing in 1 N sodium hydroxide at 100° C. for 10 minutes. The esterified principle was found to be 10% as active as the parent acid and apparently insoluble in water. The results obtained are given in Table VIII.

Table VIII

Reaction time (minutes)	1	3	10	20	30
Percent esterification	50	55	60	80	85

Distillation of the n-butyl ester.— By subjecting material (potency = 200) containing the n-butyl ester of the factor to 100° C. and 0.5 mm. of mercury for 2 hours, 20% of the activity was found to have distilled. Eighty percent of the activity sublimed when more of the same material was subjected to 150° C. and 10^{-6} mm. of mercury for 22 hours in a sublimation apparatus.

Concentration of the Factor

Preliminary Concentration Studies.

On the basis of experience gained in preliminary trials the following concentration procedure was carried out. Two hundred grams of liver fraction B dissolved in 7 l. of water was clarified by filtering through kieselguhr. Fifty grams of Darco G-60 was added, stirred for 15 minutes and filtered. The filtrate was discarded. The residue was boiled 10 minutes under reflux with 1 l. of 14% aqueous ammonia. After filtration, the filtrate was again discarded, the residue being eluted in 1 l. of 10% aqueous aniline solution by stirring for 10 minutes at room temperature. The residue was discarded and, following removal of the aniline by shaking with ether, the filtrate was made 7% in ammonia content. Ten grams of Darco G-60 was added, stirred 10 minutes, and filtered. The filtrate was discarded and the residue was eluted in 200 ml. of 10% aniline as in the previous step. After removal of the aniline following filtration, the filtrate was adjusted to pH 3.0 and extracted with three 100 ml. portions of n-butyl alcohol. The butyl alcohol which dissolved in the aqueous layer was removed by shaking it with ether, the butyl alcohol layer being discarded. To the resulting solution 0.2 g. of Darco G-60 was added. This was stirred for 10 minutes and filtered, discarding the filtrate. The residue was suspended in 40 ml. of 10% aniline and stirred for 10 minutes. After filtration and removal of the aniline, the filtrate was found to have a potency of about 600. The yield of activity in this concentrate compared to the starting material was, however, only 3%.

Concentration of the Factor from "Activated" Charcoal.

A large quantity of Darco G-60 which had been treated with liver fraction B by the Wilson Laboratories of Chicago, Illinois, was kept on hand and used as a source of the factor. The directions for its preparation which had been sent to the Wilson Laboratories follow:[7]

> Dissolve the amount of R. E. 256* equivalent to 100 lbs. of dry material in 230 gallons of water. (Hot water can be used in making solution if necessary.)
>
> Adjust the pH to 7 ± 0.1 with NaOH.
>
> Clarify thoroughly (Kieselguhr or filter cell may be used).
>
> Stir with 5 lbs. of Darco G-60 for 30 minutes at room temperature. Filter off the charcoal, wash it with about 5 gallons of water (neutral), dry at room temperature, mix, weigh and ship to us labelled:
>
> Activated Charcoal R. E. 256 No. 1+
> Net Weight_____
>
> Adjust the pH of the filtrate to 4.5-5.0, using sulfuric acid. Stir the filtrate with 80 lbs. of Darco G-60 at room temperature for 30 minutes. Filter off charcoal, wash with water thoroughly (neutral), dry at room temperature, mix thoroughly, weigh and ship to us labelled:
>
> Activated Charcoal R. E. 256 No. 2
> Net Weight_____
>
> Filter cel in small amounts may be used if necessary to aid in filtering off activated charcoals. Bacterial decomposition must, of course, be avoided throughout the process.

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[7] Excerpt from a letter to Wilson Laboratories, October 14, 1941. See letter Oct 16, '41

*The manufacturer's designation for a particular supply of liver fraction B.

+This first batch of charcoal was used in this laboratory for another purpose.

Batches of the activated charcoal from the second adsorption (No. 2 above) were worked up 1 kg. at a time to a concentrate having a potency of 110. The procedure used for obtaining this concentrate follows: One kilogram of the active charcoal was suspended in 6 l. of 14% aqueous ammonia solution and boiled under reflux for 10 minutes. The charcoal was filtered off and washed while hot, the filtrate being discarded. The residue was boiled under reflux for 10 minutes in 3 l. of 10% aqueous aniline solution and filtered. The filtrate was saved and the residue was re-eluted by boiling under reflux for 10 minutes in 3 l. of 10% aniline solution, to which 100 ml. of concentrated sulfuric acid was added. After filtration and washing, the residue was discarded and the alkalinity of the filtrate was adjusted to correspond to that of the filtrate which was saved. The two filtrates were then combined and shaken with ether to remove the aniline. To the resulting solution concentrated sulfuric acid was added to bring its pH to 3.0-3.1. The small amount of precipitate which formed was filtered off and 30 g. of Darco G-60 was added. After stirring the mixture for 10 minutes it was filtered, discarding the filtrate. The charcoal was eluted by boiling under reflux for 10 minutes in 300 ml. of 10% aniline solution. Filtration of the mixture was followed by a second elution of the residue, performed exactly as the first. The residue was then discarded. Removal of the aniline in the combined filtrates was accomplished by washing it with ether. The aqueous solution was evaporated to a thick syrup on the steam bath and to dryness in a vacuum dessicator. The resulting mass was finely pulverized and extracted with 200 ml. of 1:1 absolute

ethyl alochol and dioxane solution for 4 hours in a soxhlet extractor. The brown solid which resulted upon evaporating the solvents in the filtrate was further concentrated as described below.

Figure 2 gives yields and potencies for each step in the procedure just described.

Further concentration by precipitation with barium hydroxide.— To 2 ml. of an aqueous solution containing 0.5 g. of solids from the soxhlet extraction a saturated solution of barium hydroxide was added until it was neutral to litmus. The precipitate which formed was centrifuged down and washed twice with 2 ml. portions of water. After evaporating the combined supernatant liquid and washings to 2 ml., 15 ml. of 95% ethyl alcohol was added. The resulting precipitate was separated from the mother liquor by centrifugation and was titurated three times with 2 ml. portions of 95% ethyl alcohol.

Yields and potencies are given in Figure 3.

Further concentration of material from the soxhlet extraction by esterification, and distillation.— From 3 kg. stock charcoal there resulted 15 g. of material from the soxhlet extractions which was dissolved in 300 ml. of n-butyl alcohol. To this was added 0.9 ml. of concentrated sulfuric acid. The solution was then boiled under reflux for 30 minutes, and then was evaporated on the steam bath to about 40 ml. Two hundred milliliters of chloroform was added and the resulting solution was shaken 3 times with 100 ml. portions of water. After allowing the chloroform solution to stand overnight in a vacuum dessicator over calcium chloride it was poured through a 1.5 cm. column of

aluminum oxide 15 cm. high. The chloroform was evaporated off and the residue was subjected to distillation at 150° C. and 10^{-6} mm. of mercury for 1 hr.

Yields and potencies are given in Figure 4.

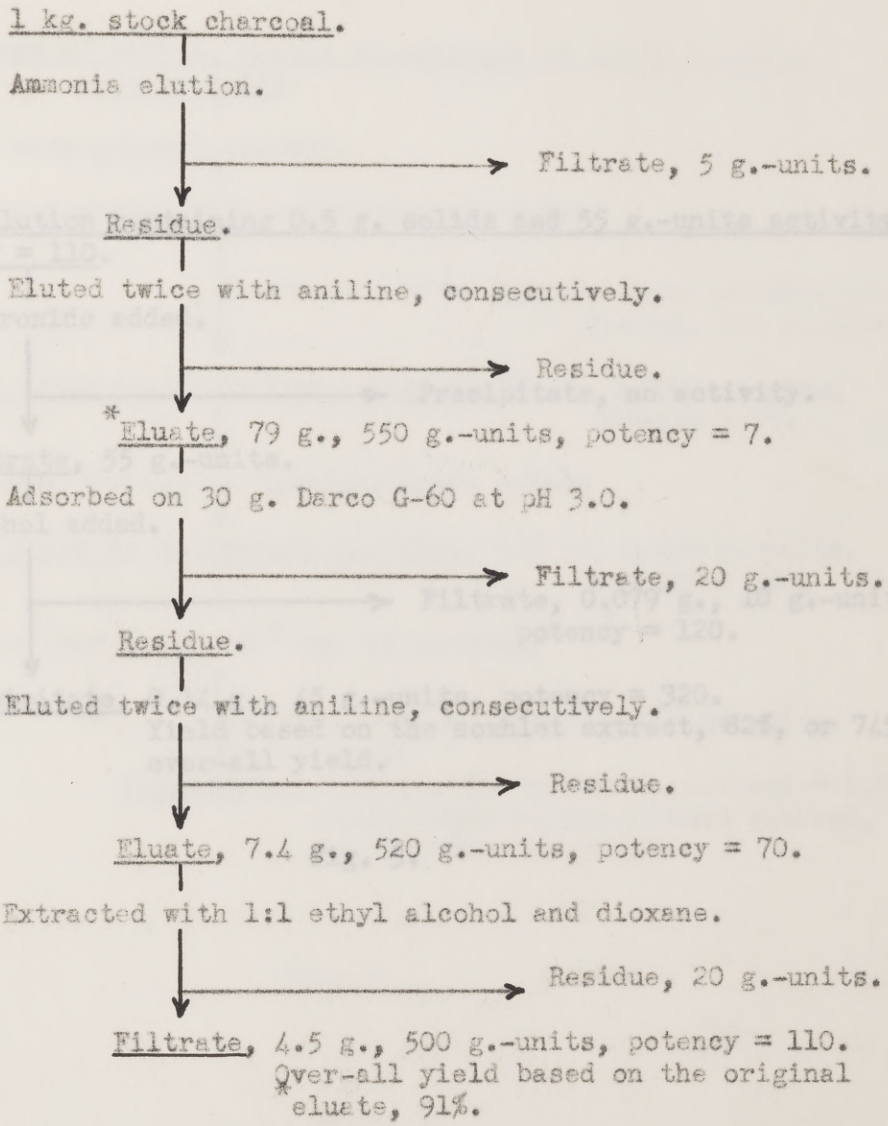
1 kg. stock charcoal.
Ammonia elution.
Filtrate, 5 g.-units.
Residue.
Eluted twice with aniline, consecutively.
Residue.
*Eluate, 79 g., 550 g.-units, potency = 7.
Adsorbed on 30 g. Darco G-60 at pH 3.0.
Filtrate, 20 g.-units.
Residue.
Eluted twice with aniline, consecutively.
Residue.
Eluate, 7.4 g., 520 g.-units, potency = 70.
Extracted with 1:1 ethyl alcohol and dioxane.
Residue, 20 g.-units.
Filtrate, 4.5 g., 500 g.-units, potency = 110.
Over-all yield based on the original *eluate, 91%.

Fig. 2.

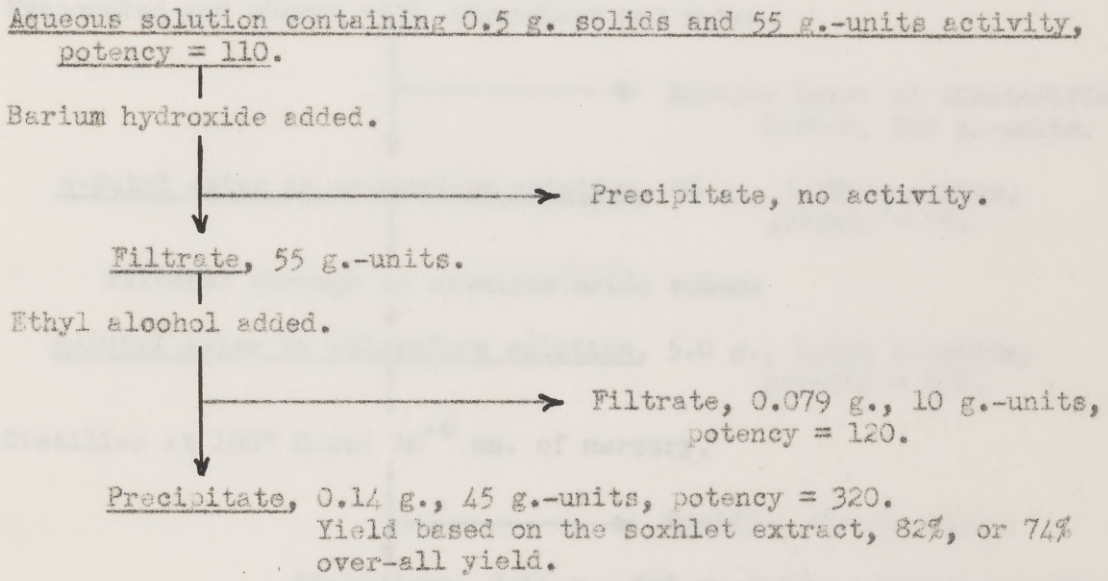
Aqueous solution containing 0.5 g. solids and 55 g.-units activity, potency = 110.
Barium hydroxide added.
Precipitate, no activity.
Filtrate, 55 g.-units.
Ethyl alcohol added.
Filtrate, 0.079 g., 10 g.-units, potency = 120.
Precipitate, 0.14 g., 45 g.-units, potency = 320.
Yield based on the soxhlet extract, 82%, or 74% over-all yield.

Fig. 3.

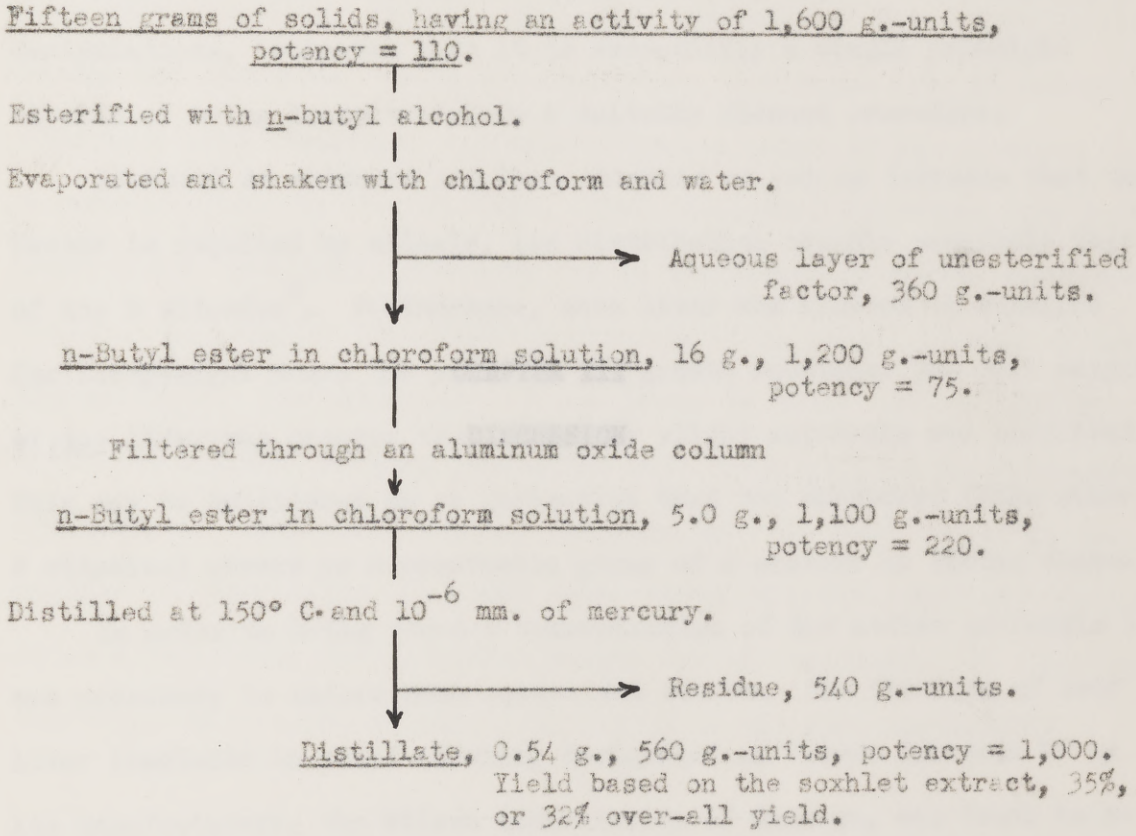
Fifteen grams of solids, having an activity of 1,600 g.-units, potency = 110.
Esterified with n-butyl alcohol.
Evaporated and shaken with chloroform and water.
Aqueous layer of unesterified factor, 360 g.-units.
n-Butyl ester in chloroform solution, 16 g., 1,200 g.-units, potency = 75.
Filtered through an aluminum oxide column
n-Butyl ester in chloroform solution, 5.0 g., 1,100 g.-units, potency = 220.
Distilled at 150° C. and 10^{-6} mm. of mercury.
Residue, 540 g.-units.
Distillate, 0.54 g., 560 g.-units, potency = 1,000.
Yield based on the soxhlet extract, 35%, or 32% over-all yield.

Fig. 4.

CHAPTER III

DISCUSSION

DISCUSSION

The behavior of the growth promoting agent during the concentration procedure involving adsorptions, elutions, extractions, and other manipulations, indicates that it is essentially a single principle capable of being concentrated by a suitably planned procedure.

Although no evidence has been obtained as yet to indicate that the factor is required by animals, its distribution roughly parallels that of the B vitamins[8]. Furthermore, when liver was allowed to autolyze for forty-eight hours the yield of the growth substance per unit weight of the liver was greater than when only slight autolysis was permitted. This may be considered as an indication that the substance (like other B vitamins) occurs as a prosthetic group of a protein in living tissue.

In order to bring about a concentration of the active principle it was necessary to choose some convenient source. The fraction of beef liver insoluble in ethyl alcohol, designated as "liver fraction B" by its manufacturers, the Wilson Laboratories of Chicago, was found to be the most suitable source material among those investigated. It is a relatively rich source, and it is readily available and inexpensive.

The first step in the concentration procedure, involving adsorption on active charcoal, could well be accomplished in a factory equipped for this type of work, and was carried out by the Wilson Laboratories. Although several procedures were developed for further concentration, the

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[8] University of Texas Publication, op. cit.

one found most suitable involved another adsorption on charcoal followed by extraction with organic solvents, esterification, filtration through alumina and distillation.

During the course of developing the procedure, several inferences were drawn as to the probable molecular structure of the active principle. Fractional electrical transport migration, adsorption from an acidic solution, salt formation, and esterification point to the presence of a carboxyl group in the molecule. That the active substance undergoes destruction upon acid hydrolysis and is unaffected by sodium hydroxide solution may suggest the possible presence of an acetal linkage within the molecule. The solubility of the factor in water and its insolubility in organic solvents immiscible with water hint at the presence of at least one other polar group. The fact that the butyl ester is insoluble in water while the ethyl ester shows definite solubility indicates a relatively low molecular weight for the compound. The fact that the butyl ester could be distilled points to the same conclusion.

CHAPTER IV

SUMMARY

SUMMARY

1. The presence of an active principle in yeast and liver extracts that causes the rapid growth of Leuconostoc mesenteroides has been noted. It is not identical to any of the known nutrilites.

2. The distribution of the nutrilite as well as some of its chemical and physical properties have been determined.

3. Procedures for concentrating the nutrilite from a liver extract have been developed.

4. Clues as to the molecular structure of the nutrilite are noted, and its similarity to the B vitamins is discussed.

CHAPTER V

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